Assessment Run 21 2007
CD117

The slide to be stained for CD117 comprised:
1. Appendix, 2. Liver, 3-4. Gastrointestinal stromal tumour (GIST), 5. Desmoid tumour.
All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD117 staining as optimal included:

- A strong and distinct predominantly membranous but also cytoplasmic staining of the Cajal cells in the appendiceal muscularis propria.
- A moderate to strong, distinct staining of the majority of the neoplastic cells of the two GISTs.
- A negative reaction of the desmoid tumour.
- A strong staining of mast cells in all specimens.
- A generally negative staining of all other cells (in particular smooth muscle cells). However, a weak to moderate, diffuse or granulated cytoplasmic reaction in the appendiceal enterocytes and liver cells was accepted, as it did not interfere with the interpretation of the specific staining.

118 laboratories submitted stains. At the assessment 42 achieved optimal marks (36 %), 50 good (42 %), 16 borderline (14 %) and 10 poor marks (8 %).

The following Abs were used:
mAb clone **T595** (Novocastra, n=1)
rmAb clone **9.7** (Ventana, n=5)
pAb **A4502** (Dako, n=109)
pAb **CMC766** (Cell Marque, n=1)
pAb **RB-1518** (NeoMarkers, n=1)
pAb **sc-168** (Santa Cruz, n=1)

Optimal staining for CD117 in this assessment was only obtained with the pAb clone **A4502** (42 out of 109).

A4502: All the protocols giving an optimal result were based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (29/61)*, Cell Conditioning 1 (BenchMark, Ventana) (6/19), Citrate pH 6 (2/12), Bond Epitope Retrieval Solution 2 (Bond, Leica-microsystems) (1/3), Bond Epitope Retrieval Solution 1 (Bond, Leica-microsystems) (1/1), EDTA/EGTA pH 8 (1/6) or 1mM EDTA pH9 (1/1) as heating buffer. The mAb was typically diluted in the range of 1:50 – 1:1.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 79 out of 97 (81 %) laboratories produced a sufficient staining (optimal or good).
In 6 protocols, HIER was omitted. Of these, 3 were marked as good and 3 as borderline.
* (number of optimal results/number of laboratories using this buffer)

The most frequent causes of insufficient staining were:
- Less successful primary antibody
- Too low concentration of pAb A4502
- Too high concentration of pAb A4502
- Insufficient epitope retrieval - HIER in citrate pH 6.0 or EDTA/EGTA pH8 with too short heating time, or omission of HIER.

In this assessment and in concordance with the results in the previous run 14 2005, the prevalent features of an insufficient staining were either a weak/false negative or a false positive reaction in the specimens. The weak/false negative reaction was primarily seen in the appendiceal Cajal cells and the two GISTs (typically appearing with too low concentration of primary Ab or insufficient/missing HIER). The false positive reaction was typically seen in the smooth muscle cells in the appendix (typically appearing with protocols using a too high concentration of the primary Ab).
In 5 laboratories a distinct reaction of the neoplastic cells of the desmoid tumour was observed. The protocol settings in these 5 laboratories were identical to the laboratories not demonstrating the neoplastic cells in the desmoid tumour. This aberrant reaction in the desmoid was also noticed in run 14 and could not be explained at that time. However, it has now been revealed that in 3 of the 5 laboratories, the same A4502 lot OHO12A was

used in the two runs with identical reactions in the desmoids. In a re-test in the NordiQC lab the reaction pattern with lot OHO12A has been confirmed.
Appendix is an appropriate control for CD117: The Cajal cells must show a distinct reaction, while the smooth muscle cells in muscularis propria must be negative.

CD117 was also assessed in runs 7 and 14. The proportion of insufficient results has been reduced from 38 % in run 7 2003 to 22 % in the present run. Specific recommendations have been given to 35 laboratories with insufficient results in the two previous runs. 21 laboratories followed the recommendations and 15 of these (71%) improved. Six did not follow the recommendations and 2 of these (33%) improved. Two laboratories changed their entire system resulting in a sufficient result.

Conclusion
pAb A4502 is an appropriate CD117 Ab for the identification of GIST.
HIER, preferable in an alkaline buffer, is mandatory to obtain an optimal result. Concentration of the primary Ab should be carefully calibrated.
Appendix is an appropriate control for CD117: The Cajal cells must show a distinct reaction, while the smooth muscle cells in muscularis propria are negative.
It has to be further elucidated if a lot-to-lot variation can cause an aberrant CD117 reaction in desmoids and similar tumours.

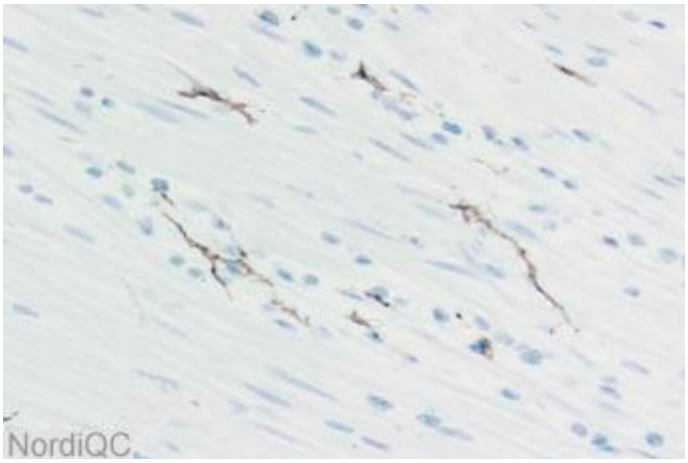

Fig. 1a
Optimal staining of CD117 (pAb A4502) The Cajal cells in the appendiceal muscularis propria are distinctively stained. The smooth muscle cells are unstained.

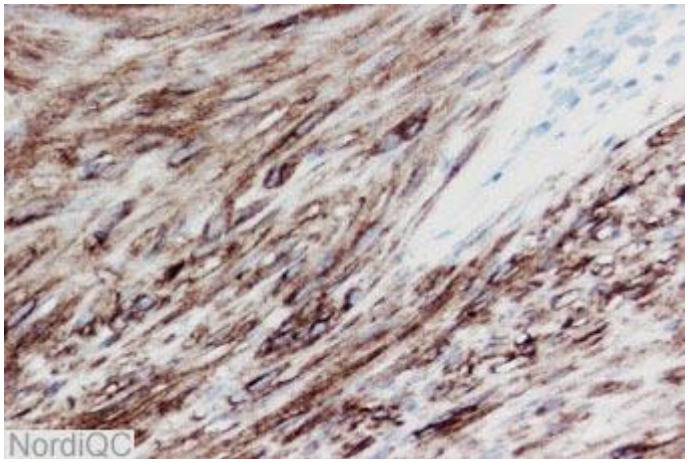

Fig. 1b
Optimal staining of CD117 in a GIST (same protocol as in Fig. 1a). Virtually all the neoplastic cells show a strong cytoplasmic reaction with membrane enhancement and focally a dot-like reaction.

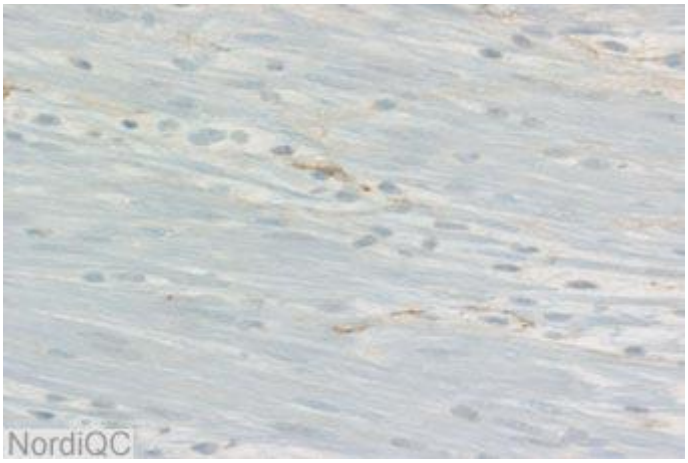

Fig. 2a
Insufficient staining of CD117 (pAb A4502). The Cajal cells in the appendiceal muscularis propria are only weakly demonstrated.

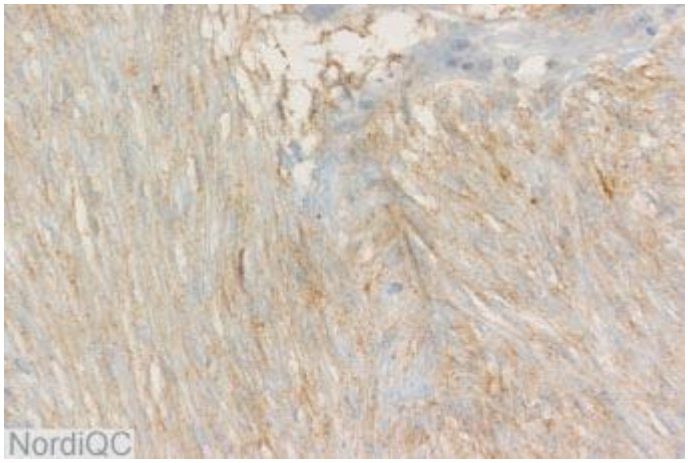

Fig. 2b
Insufficient staining CD117 in a GIST – same protocol as in Fig 2a. The neoplastic cells only show a weak diffuse staining – compare with Fig 1b – same tumour.

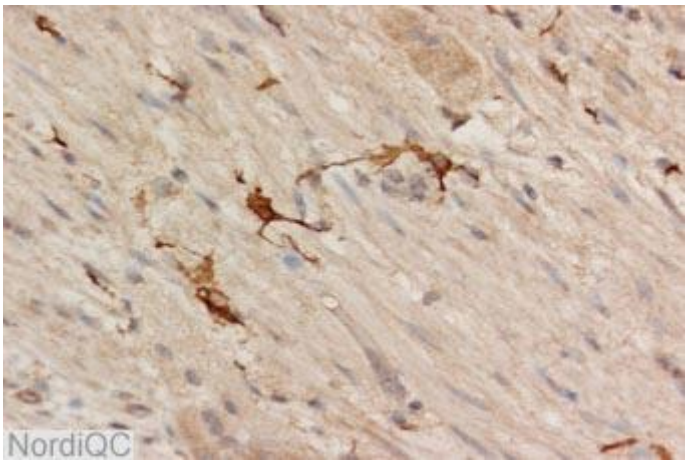

Fig. 3a
Insufficient staining of CD117 (pAb A4502) The Cajal cells in the appendiceal muscularis propria are demonstrated, but both the ganglion cells and smooth muscle cells show a false positive staining. This reaction is typically seen in protocols using a too high concentration of the primary Ab.

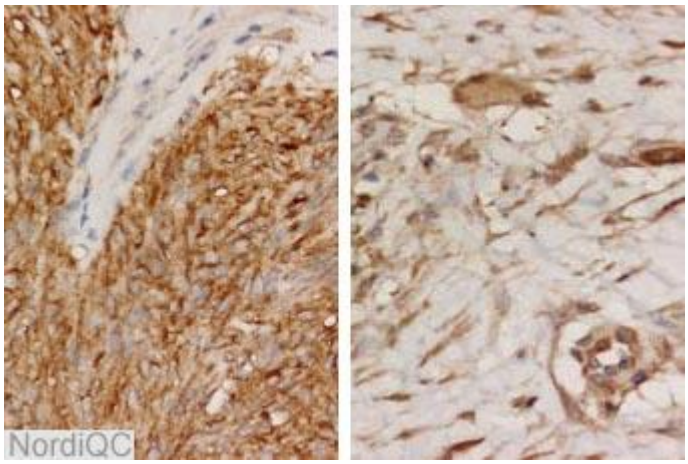

Fig. 3b
Left: Insufficient staining CD117 in a GIST – same protocol as in Fig 3a. The neoplastic cells are demonstrated, but compare with Fig. 3b right.
Right: Insufficient staining of the desmoid tumour – all structures are stained. Same protocol used as in Fig. 3a.

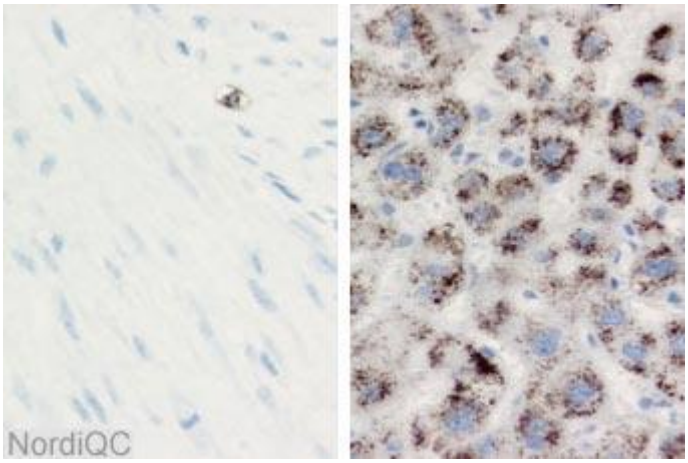

Fig. 4a
Staining of CD117 using the pAb A4502, lot no. 4938. Same protocol as used in Fig. 1a and 1b – HIER in Tris-EDTA pH 9.0 and Ab diluted 1:400.
Left: The desmoid tumour is negative and only mast cells are demonstrated.
Right: The liver cells show a granular cytoplasmic reaction (seen with several A4502 lots).

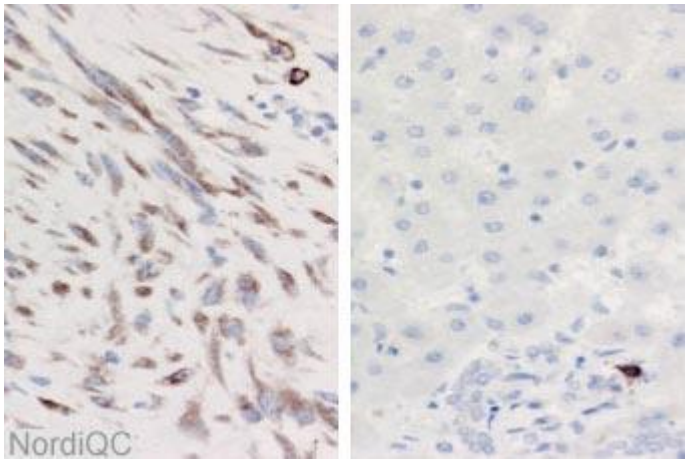

Fig. 4b
Staining of CD117 using the pAb A4502, lot no. OHO12A. Same protocol as used in Fig. 4a.
Left: The desmoid tumour shows a distinct reaction.
Right: The liver cells are all negative and only mast cells are demonstrated. This pattern was also seen with the lot 1J019A (one lab), but not with lot no. 4938.

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